Numerical dating algorithms of amino acid racemization ratios from continental ostracodes. Application to the Guadix-Baza Basin (southern Spain)

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Abstract

Age calculation algorithms for the D/L ratios of five amino acids (isoleucine, leucine, aspartic acid, phenylalanine and glutamic acid) analysed in continental ostracodes were determined for southern and central Iberian Peninsula, and allow the numerical dating of deposits in the Mediterranean area since Lower Pleistocene time to present. In order to obtain more accurate results for young samples, other algorithms were calculated for aspartic acid, phenylalanine and glutamic acid. Using these algorithms, together with paleomagnetism, the chronostratigraphy of the "composite-stratotype-section" of the east domain of the Guadix-Baza Basin that covers most of the Pleistocene (from the Plio/Pleistocene boundary to 279 ± 77 ka) has been obtained. Ostracodes represent a formidable tool for amino acid racemization dating purposes in view of their abundance, valves mineralogy and the high degree of preservation of amino acids within their caparaces, even in old (Early Pleistocene) samples. These results suggest that the range of the amino acid racemization dating method in the Iberian Peninsula is older than 1.1 Ma.

1. Introduction

In recent years there has been a proliferation of palaeoenvironmental studies that use various techniques to characterize changes in climate and environmental processes in the past. However, one of the greatest challenges confronting palaeoenvironmental and palaeoclimatological studies is the need to place the observations on a chronostratigraphical scale. These reconstructions need to be achieved by a huge number of dating methods depending on the time scale, e.g. ^{210}Pb, ^{14}C or U/Th. Besides them, there is the amino acid racemization method, which can be applied to a large number of materials, including mollusk and ostracode shells. The amino acid racemization method is especially useful for the age range of 10^5–10^6 yr BP, which is completely beyond the range of radiocarbon method and partially beyond that of U/Th method. Amino acid racemization has also been employed in dating Holocene materials, as well as younger samples from the last century or decades (e.g. Goodfriend, 1991, 1992; Goodfriend et al., 1992, 1995) and, even, in forensic determinations of age of death (Othani et al., 1998).

However, the amino acid racemization method is not a numerical dating method in isolation. There are two general approaches to calculate the age of a sample. The first one is based on the effects of time and temperature on the amino acid racemization/epimerization process, which may be determined in "high"-temperature laboratory experiments. These data together with the kinetic model equation can provide the age of a sample if its temperature history is known. The second approach consists on the calibration of D/L ratios with

numerical datings in order to obtain age calculation equations.

Likewise, the racemization process is both genus and temperature dependent, so these algorithms can only be calculated from samples located in areas with the same thermal history.

Our experience (Ortiz, 2000; Ortiz et al., 2002) indicates that ostracodes have significant characteristics that make them particularly useful for amino acid racemization/epimerization dating:

(1) Ostracode valves are mainly composed of low-magnesium-calcite (Sohn, 1958; Cadot and Kaesler, 1977; Bordegat, 1979, 1985) and initially racemize faster than gastopods (Ortiz et al., 2002).
(2) In most cases, ostracodes are abundant and the only fossil fauna present in beds, so gastropods or bivalves cannot be used to obtain a complete and accurate amino acid chronology for a certain area.
(3) The excellent preservation of amino acids in ostracode valves means that only a small sample size (10–20 mg) is required for analysis by gas chromatography (GC), much less than for other organisms (e.g. molluscs 80 mg). Using reverse phase high performance liquid chromatography (HPLC), it is possible to analyse even a single ostracode valve (cf. Kaufman, 2000).
(4) In a single GC analysis sample, there are typically between 1500 and 2000 ostracode valves, so the standard error or variance is low given the statistical significance of the sample size.

At the Biomolecular Stratigraphy Laboratory (BSL), we are studying the Pleistocene paleoenvironmental evolution of different areas in Spain. Within the geological record of three of them (Fig. 1): Guadix-Baza continental Basin (Granada, Andalusia, southern Spain), Padul peat bog (Granada, Andalusia, southern Spain) and the travertine fluvial terraces of Priego area (Cuenca, central Spain), the ostracode species *Cyprideis torosa* (Jones), especially in the first zone, and *Herpetocypris reptans* (Baird) are very common. Some beds containing these species were suitable to be dated by numerical dating methods such as ^{14}C and U/Th; in other cases, paleomagnetism (Oms et al., 1994; Ortiz, 2000) was used to assign an age to some samples and, finally, some deposits were previously dated by the amino acid racemization method applied to continental gastropods (Torres et al., 1995, 1997; Ortiz et al., 2000). Therefore, the application of these procedures make it possible to establish age calibration models.

The use of samples from Guadix-Baza Basin, Padul peat bog and Priego fluvial travertine terraces all together, which are located in the Mediterranean climatic zone of the Iberian Peninsula, is justified by the fact that a similar thermal history can be inferred for these areas given their similar CMAT: 12–14°C (Current Mean Annual Temperature) (cf. Torres et al., 1994, 1997).

Finally, we apply these new algorithms to obtain the chronostratigraphy of a 356-m-thick "composite-stratotype-section" of the east domain of the Guadix-Baza Basin, ranging from the Plio/Pleistocene boundary to the upper part of the Middle Pleistocene. This basin is particularly interesting because it is one of the few zones in Europe, together with Lac du Bouchet and Praclaux in France (Beaulie and Reille, 1995; Reille and Beaulie, 1995), Valle di Castiglione in Italy (Follieri et al., 1998) and Ioannina in Greece (Tzedakis, 1994), among others, where almost continuous sedimentation took place during most of the Quaternary period. Palaeoclimatological and palaeoenvironmental changes during the Pleistocene can therefore be deduced from paleobiological and geochemical studies. Similarly, several vertebrate paleontological sites have been located and studied in this area; one of them, named Venta Micena, with controversial human remains.

Previous workers have established chronostratigraphic frameworks for the Guadix-Baza Basin, based on paleontological data in the absence of numerical dating (Agustí, 1986; Anadón et al., 1987; Alberdi et al., 1989). The purpose of this study was therefore to obtain the numerical chronostratigraphy of the basin.

2. Geographical setting, geology and stratigraphy

2.1. Priego area

Priego (Cuenca, central Spain) is situated within the Iberian Range where three rivers have built a wide system of travertine terraces during the Pleistocene (Fig. 1). Seven travertine terrace levels have been identified (Torres et al., 1994). Downstream, river terraces are made of clastic sediments.

2.2. Padul

The Padul peat bog is located 20 km south of Granada city in Andalusia, southern Spain (Fig. 1). It consists of a 4 km^2 tectonic trough in the form of an endorheic basin, surrounded by the mountains of the Betic Range, placed 720 m above sea level. In 1997 a new 103-m-long borehole (latitude: 37°01′1″, longitude: 3°36′7″, elevation: 714 m) was drilled to study the pollen assemblages as well as the stable isotopes and biomarkers of sediments in order to reconstruct the Pleistocene paleoenvironmental history of the southern part of the Iberian Peninsula. In Fig. 2 we have represented the stratigraphical record of the Padul borehole core (for a detailed description, see Nestares and Torres, 1998).

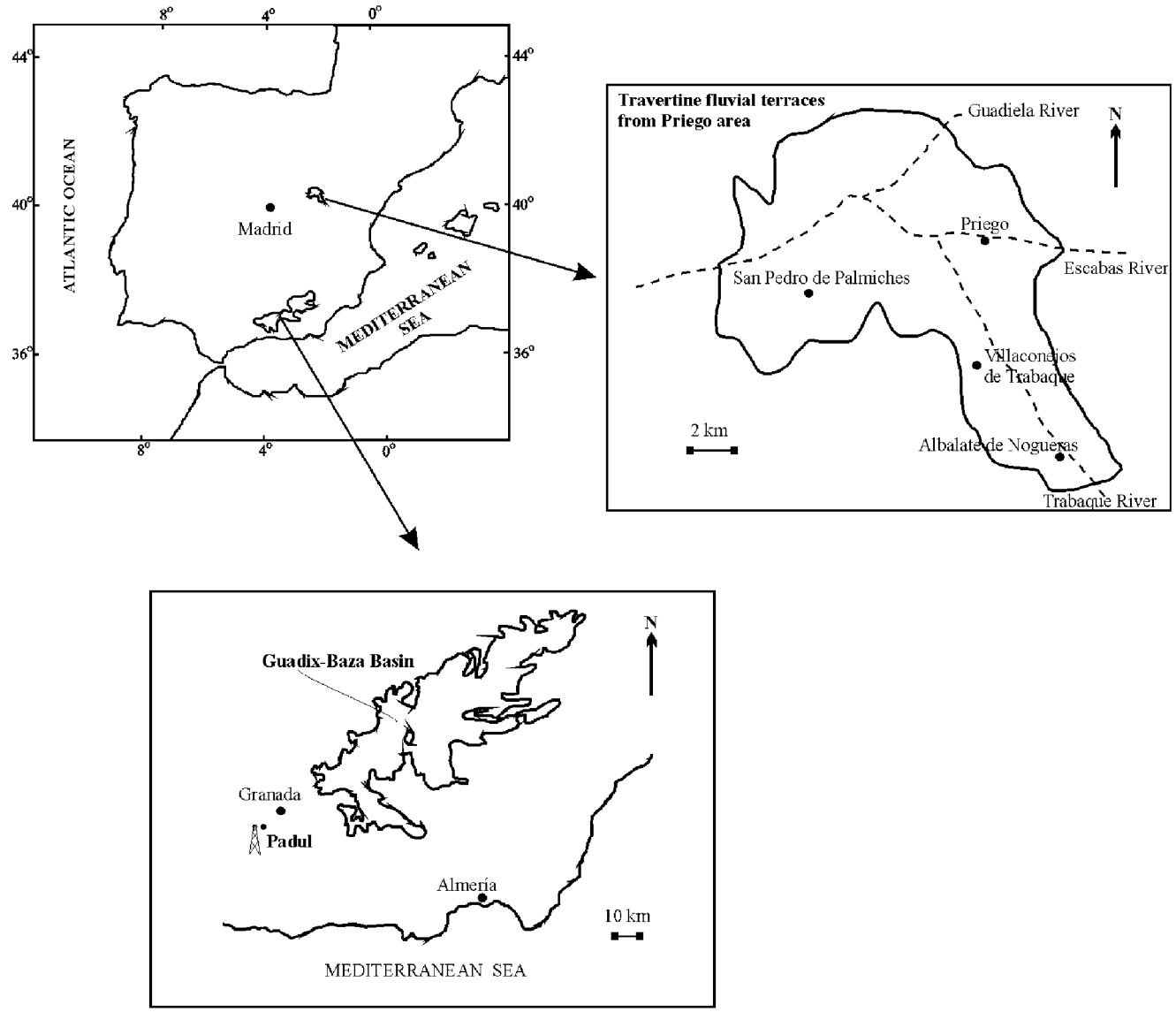

Fig. 1. Geographical location of Guadix-Baza Basin, Padul peat Bog and Priego area.

2.3. *Guadix-Baza Basin*

Guadix-Baza Basin is a "basin and range" zone that covered a large extension, aproximately 4500 km^2. It has an irregular shape with its major axis oriented SW–NE. Its origin is related to the Alpine Orogeny (Soria, 1993) which affected Mesozoic and Cainozoic rocks within the region. Later, during the Upper Tortonian the sedimentary conditions changed to a continental regime.

The basin can be understood within a centripetal depositional model, that is, coarse grained alluvial fans at the foot of mountain ranges, which gradually pass into a system of channels that flowed out to a central system of small saline lakes distributed in a mosaic pattern with sedimentation of gypsiferous lutites, gypsiferous sands, gypsum and, sometimes, decimetrical lutite beds with displacive gypsum crystals (Torres et al., 2003). At the end of Middle Pleistocene, erosive processes began, and the current fluvial system was established.

In the eastern part of Guadix-Baza Basin (GBE) we have established a 356-m-thick "composite-stratotype-section" (Fig. 3), referred to as CBS, which is representative of the depositional history of the basin from the Pliocene–Pleistocene boundary to the upper part of the Middle Pleistocene. It is composed of two sub-sections: Cortes de Baza (CTB) and Norte de Orce (CNOR) sections. According to magnetostratigraphic studies of the geological record of this Basin (Oms et al., 1994; Ortiz, 2000), supported by paleontological data (Agustí, 1986), three important palaeomagnetic events have been reported: the end of the Olduvai chron (ca 1.77 Ma), the Matuyama/Brunhes boundary (ca 780 ka) and a short reverse polarity event which can be correlated to either Emperor or Lake Biwa III excursions, dated at ca 419 and ca 412 ka (Cande and Kent,

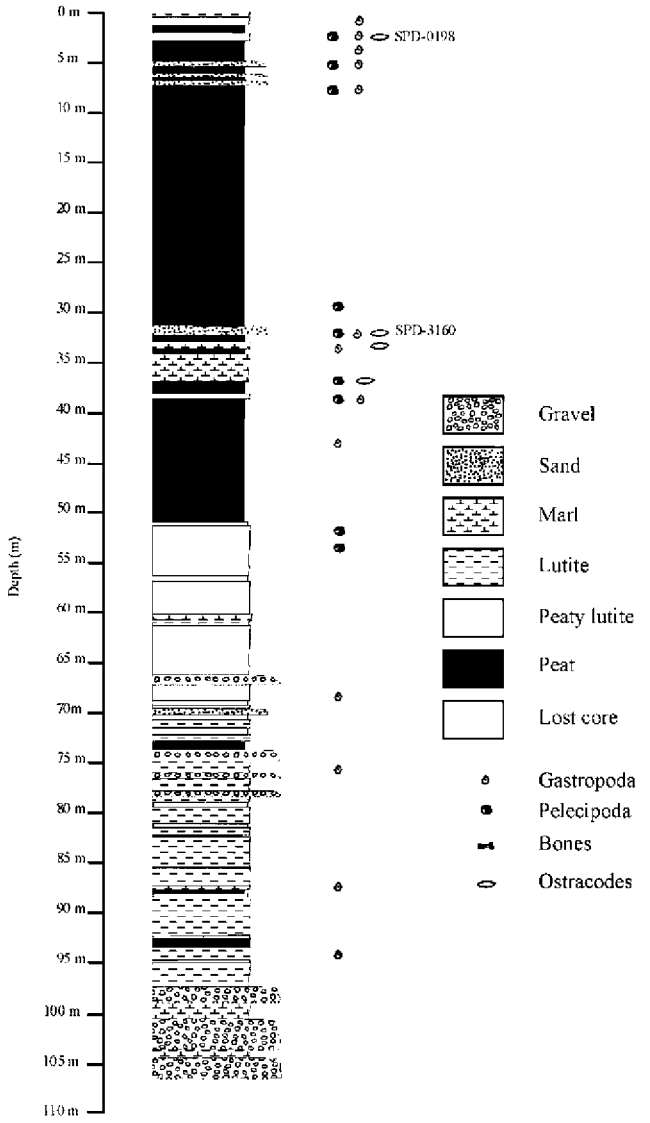

Fig. 2. Stratigraphy of Padul peat bog borehole (Nestares and Torres, 1998).

1995) respectively. We refer the sampled horizons of this section according to their position, in meters, from bottom to top (e.g. sampled level CBS-125 corresponds to 125 m).

A more detailed description of the stratigraphic section and the stratigraphy and paleontology of this basin may be found in Ortiz (2000) and Torres et al. (2003).

3. Materials and methods

For the calculation of the dating algorithms for amino acid racemization ratios analysed in continental ostracodes, we have employed samples of 10 localities (Table 1). For the calibration of the algorithms for time zero, ostracodes recovered from the Cabo de Gata salines (CGS) (Andalusia, Spain) were also used.

Two localities, Priego-6 and Priego-7 (PR-6 and PR-7), were travertine fluvial terraces located in the Priego area (Central Spain) and were previously dated by the U/Th method (Torres et al., 1994).

Two of them, SPD-0198 (stratigraphic level from a depth of 198 cm) and SPD-3160 (3160 cm deep), were taken from the Padul borehole core (SPD) for radiocarbon and U/Th dating respectively.

The other six localities came from the Guadix-Baza Basin, three of them were collected in paleontological sites, such as Fuente Amarga (FA), Cúllar-Baza (CB) and Venta Micena (VM), dated by Ortiz et al. (2000) using the amino acid racemization method applied to gastropods. We also used three stratigraphic horizons from the Guadix-Baza stratigraphic composite stratotype section: CBS-253, CBS-268 and CBS-323. The age of these horizons was established by paleomagnetism analysis (Ortiz, 2000).

For the age determination of the Guadix-Baza Basin stratigraphic section we selected ostracodes at different horizons: CBS-206, CBS-223, CBS-228, CBS-281, CBS-303, CBS-314, CBS-327, CBS-330 and CBS-352.

3.1. U/Th dating

For U/Th dating of SPD-3160 we selected and prepared pure peat using the procedure developed by Vogel and Kronfeld (1980). Both SPD-3160 and the Priego samples (PR-6 and PR-7) were analysed by one of us (R.J.).

The procedure used for chemical separation is based on that developed by Bischoff and Fitzpatrick (1991). In this procedure, the sample is totally dissolved in strong mineral acids and a radioisotope with known activity is incorporated in order to determine the efficiency of the isotope separation. The U and Th isotopes were isolated by ion-exchange chromatography and then analysed in an alpha spectrometer from Ortec with a silica barrier detector. For age calculation the program UDATE from Rosenbauer (1991) was used.

3.2. ^{14}C dating

Radiocarbon dating was undertaken on a peat of sample SPD-0198. For this purpose, e.g. 100 mg were analysed in the Instituto de Química Física Rocasolano (C.S.I.C., Madrid). Materials measured by the radiometric technique were analysed by synthesizing CO_2 from the sample to benzene, measuring for ^{14}C content in a scintillation spectrometer, and then calculating for radiocarbon age. The age was calibrated using the program CALIB from the Washington University, method B, 2 sigma (95.4% confidence intervals) (Stuiver and Reimer, 1993).

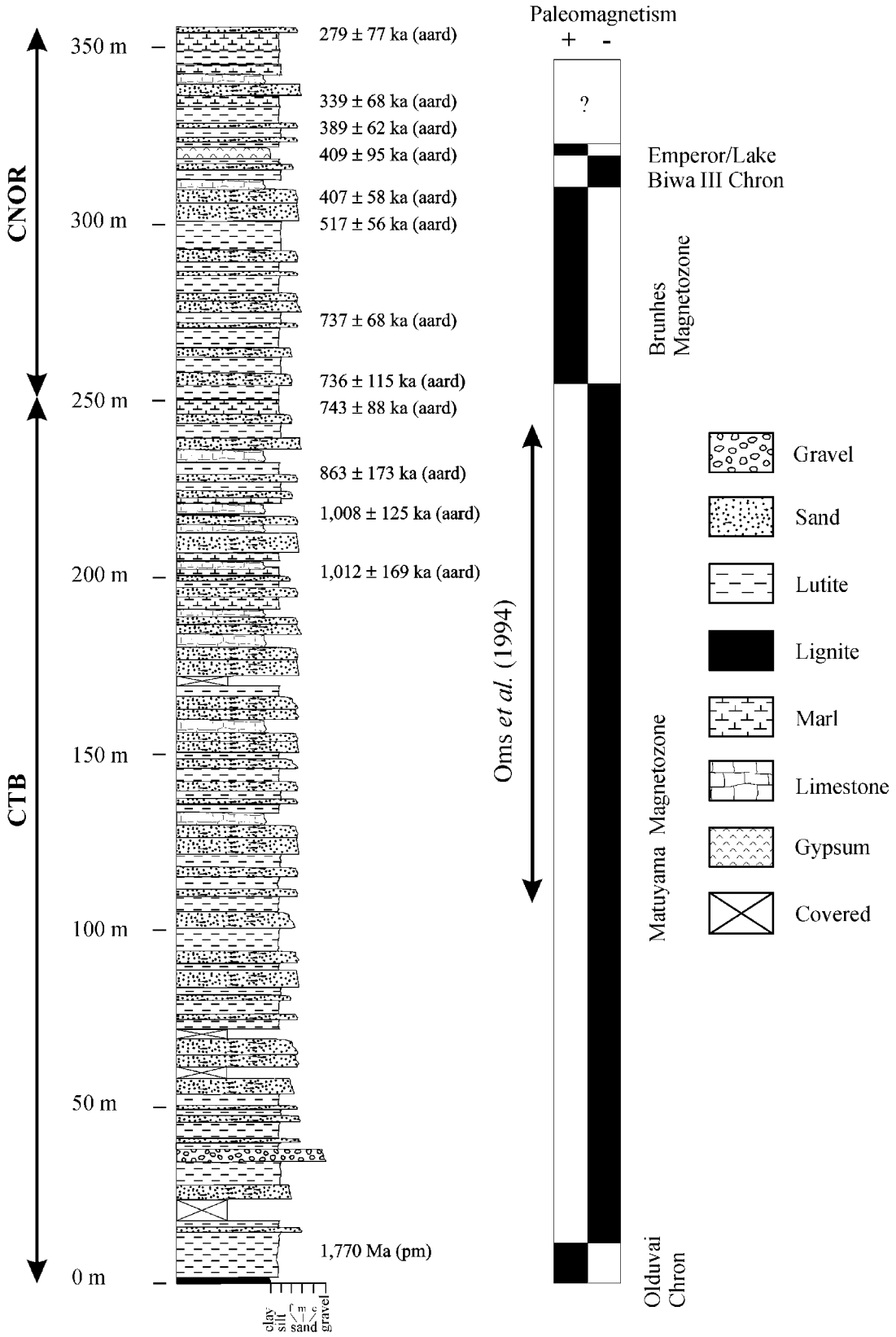

Fig. 3. Chronostratigraphy of the GBE Basin representative stratigraphic section using the amino acid racemization method and paleomagnetism. Paleomagnetism results are based on Ortiz (2000) and Oms et al. (1994).

Table 1
Geographical location of the localities

Locality	Latitude	Longitude	Elevation (m)
CGS	36°45′15″	2°12′53″	1.5
SPD-0198	37°01′1″	3°36′7″	714
PR-6	40°22′23″	2°16′32″	850
PR-7	40°22′23″	2°19′28″	820
SDP-3160	37°01′1″	3°36′7″	714
FA	37°46′7″	2°35′12″	880
CBS-323	37°47′4″	2°29′0″	1004
CB	37°34′10″	2°33′50″	940
CBS-268	37°43′59″	2°30′23″	942
CBS-253	37°38′57″	2°44′50″	782
VM	37°44′8″	2°24′27″	960

3.3. *Amino acid racemization dating*

For amino acid racemization dating purposes, samples recovered in both the Guadix-Baza Basin and the Priego area were collected digging 30–50 cm deep holes to avoid surface contamination. They were recovered in cliff faces (FA, CBS-253, CBS-268, CBS-281, CBS-303, CBS-314, CBS-327, CBS-330 and CBS-352), road cut-offs (PR-6, PR-7, CBS-323, CBS-206, CBS-223 and CBS-228) and palaeontological excavation sites (CB, Venta Micena). The field campaigns were performed in summer and samples were picked when we noticed that the sediment was colder than the external air temperature. In samples from Padul peat bog core we reject the first centimeter. It is difficult to find carbonate fossil remains in peat bog sediments as a result of the dissolution (leaching) of the carbonate shells that can be caused by low pH water. In spite of this, we were able to recover ostracode shells in some beds of the core.

Samples were sieved under running water and dried at room temperature. After drying, the samples were studied under a binocular microscope to determine the faunal assemblages. Ostracodes were carefully sonicated and cleaned with water to remove the sediment contained inside their valves. Afterwards, at least 10 mg of ostracodes (ca 2000 single valves) were picked.

The sample preparation protocol is described in Goodfriend (1991) and Goodfriend and Meyer (1991) and involves:

(1) Hydrolysis which was performed under N_2 atmosphere in a mixture of 12 N HCl (2.9 μl/mg) and 6 N hydrochloric acid (100 μl) for 20 h at 100°C; later the samples were then desalted in HF and the resultant supernatant frozen and dried under vacuum.
(2) Derivatization: amino acids were derivatized in a two step process, involving first esterification with 250 μl of 3 M thionyl chloride in isopropanol for 1 h at 100°C under N_2; the samples were dried and acylated by reaction with 200 μl of trifluoroacetic acid anhydride (25% in dichloromethane) for 5 min at 100°C. Excesses derivative and solvent were evaporated under a gentle flow of nitrogen. The sample was taken up in 100 μl of *n*-hexane which was vortexed.

One to four aliquots μl were injected into a Hewlett-Packard 5890 gas chromatograph. The injection port was kept at 215°C and set for splitless mode for the first 75 s, at the beginning of which the sample was injected, and later set to split mode. We used helium as the carrier gas, at a column head pressure of 5.8 psi, and a Chirasil-L-Val fused silica column (0.39 mm × 0.25 μm × 25 m) from Chrompack. The gradients used were as follows: 50°C (1 min), heating at 40°C/min to 115°C, 12 min at 115°C, heating at 3°C/min to 190°C, 10 min at 190°C, cooling to 50°C and remaining at this temperature between runs (at 80°C if the time between runs was longer, typically overnight). The detector was an NPD set at 300°C. Integration of the peak areas was carried out using the HP PEAK96 integration program from Hewlett-Packard, which runs on a PC computer. As a laboratory routine, D/L-alanine, D/L-valine, D-alloisoleucine/L-isoleucine, D/L-proline, D/L-aspartic acid, D/L-leucine, D/L -phenylalanine and D/L-glutamic acid peaks were identified.

4. Results and discussion

The CGS contained different species of ostracodes, but we were able to isolate the necessary number of single *Cyprideis torosa* (Jones) valves to perform analysis. In samples from the Padul peat bog (SPD-0198 and SPD-3160) and Priego area (PR-6 ad PR-7) *Herpetocypris reptans* (Baird) was the only species determined, while in the whole localities from Guadix-Baza Basin *Cyprideis torosa* (Jones) was found, constituting a monospecific group in five localities (FA, CB, CBS-253, CBS-268 and CBS-323). Only at the Venta Micena (VM) paleontological site *Ilyocypris bradyi* Sars was present together with *C. torosa* (Jones).

Although monogeneric samples are necessary to reduce taxonomically controlled variability in D/L ratios, these three different ostracode genera were employed all together to establish the age calculation model. In fact, in previous studies (McCoy, 1988; Oviatt et al., 1999; Kaufman, 2000; Kaufman et al., 2001) only slight differences between D/L ratios from different ostracode genus (*Candona* and *Limnocythere*) which belong to different phylogenetic ostracode groups (Cypridacea and Cytheracea superfamilies, respectively) have been reported, as much as 0.024 in D-aIle/L-Ile values (McCoy, 1988) and 0.022 (Kaufman, 2000) or 0.048 (Kaufman et al., 2001) in D/L aspartic acid values. The analysed ostracodes of this work belong to either

one of this two superfamilies: *Herpetocypris* and *Ilyocypris* to superfamily Cypridacea and *Cyprideis* to superfamily Cytheracea.

Because of the different racemization/epimerization rates of amino acids, traditionally isoleucine epimerization ratios have been used for age calculations of relatively "old" samples, while aspartic acid, which is the amino acid that faster racemices, has been employed to date younger stratigraphic units. However, in recent times some authors have used aspartic acid D/L ratios measured in ostracodes to estimate ages of samples as old as 620 ka (Oviatt et al., 1999) or, even 1.1 Ma (Kaufman et al., 2001). Goodfriend (1991) reported strong correlations between the racemization/epimerization rates of six amino acids (alanine, proline, aspartic acid, methionine, glutamic acid and phenylalanine) measured in land snail shells from the Negev Desert (Israel). Murray-Wallace and Kimber (1993) showed also a good covariation between D/L ratios of leucine, valine and isoleucine of marine mollusks from Australia. Torres et al. (2000) observed similar variations between isoleucine and leucine D/L ratios of marine mollusks from the Spanish Mediterranean coast.

The D/L ratios of five amino acids measured in 74 analytical ostracode samples from central (Priego area) and south (Padul and Guadix-Baza Basins) Spain show strong correlations with each other (Table 2). From the 74 samples, seven included only *Hepetocypris* ostracodes (one from PR-6, two from PR-7, two from SPD-0198, two from SPD-3160), three samples consisted of valves from both *Ilyocypris* and *Cyprideis* ostracodes (all from Venta Micena site) and 64 contained only *Cyprideis* valves (FA, CB and samples from CBS). These covariance patterns were analysed by principal components analysis of the correlation matrix (Table 3). The first principal component axis (eigenvector) accounts for ca 88% of the covariation of the D/L ratios and consists of equal and positive loadings of each of the amino acids. This behaviour represents the covariation in racemization/epimerization rates among the five amino acids measured in continental ostracodes. Because of this reason we use all together the D/L ratios of the five amino acids to obtain the age calculation algorithms and the age of the samples. Based on these results we can discard anomalous D/L ratios in samples when low covariation might occur between one amino acid racemization/epimerization ratios and the others. In fact, according to Goodfriend (1991), p. 293: "...analysis of more than one amino acid provides largely redundant information on sample age".

The amino acid racemization method needs to be calibrated with previously dated samples using other methods to obtain age calculation algorithms that can be used to establish numerical datings of older deposits. Two models are commonly used for the calibration: first-order reversible kinetics (FOKs) and parabolic kinetics. Nevertheless, some other relationships between age and racemization/epimerization ratios have also been presented.

For the calibration it is necessary to have several (as much as possible) dated samples of different ages because a "non-linear" behaviour is known for the racemization process in order to obtain the "parabolic curve" of the model. When few previously dated samples are available, the calibration may be simplified considering a FOK pattern (Mitterer, 1975). However, the epimerization reaction is presumed to follow a reversible first-order kinetics only at initial stages of diagenesis, up to a D-aIle/L-Ile ratio of 0.3 (Masters and Bada, 1977; Kriausakul and Mitterer, 1980; Miller and Brigham-Grette, 1989) or 0.5 (Wehmiller and Hare, 1971; Bada and Schroeder, 1972). For fossil and pyrolized ostrich eggshells, the rate of epimerization follows FOK beyond a ratio of 0.9–1.0 (Brooks et al., 1990; Miller et al., 1991, 1992). This is because the racemization/epimerization rate eventually decreases with time, producing a non-linear relationship between

Table 2
Correlation coefficients (r) between D/L ratios of various amino acids from ostracode samples recovered in central (Priego area) and south (Padul and Guadix-Baza) Spain localities

	D-aIle/L-Ile	D/L Leu	D/L Asp	D/L Phe	D/L Glu
D-aIle/L-Ile	—	0.916	0.867	0.924	0.866
D/L Leu		—	0.887	0.923	0.852
D/L Asp			—	0.813	0.906
D/L Phe				—	0.746
D/L Glu					—

D-aIle/L-Ile: D-alloisoleucine/L-isoleucine; Leu: leucine; Asp: aspartic acid; Phe: phenylalanine; Glu: glutamic acid.
From the 74 samples, seven included only *Hepetocypris reptans* (Baird) ostracodes (from PR-6, PR-7 localities and SPD-0198 and SPD-3160 stratigraphic units), three samples consisted of *Ilyocypris bradyi* Sars and *Cyprideis torosa* (Jones) ostracodes (from VM site), and 64 contained *Cyprideis torosa* (Jones) valves (FA and CB localities and stratigraphic horizons from CBS). All correlations are statistically significant at the level of $p<0.001$.

Table 3
Principal components analysis of the correlation matrix of the D/L ratios of the five amino acids measured in ostracode samples described in Table 2, giving the first three eigenvector (principal component axis) and the proportion of the total variance in the data set

	Eigenvector 1	Eigenvector 2	Eigenvector 3
D-aIle/L-Ile	0.979	−0.074	0.074
D/L Leu	0.962	−0.117	0.071
D/L Asp	0.934	0.177	−0.311
D/L Phe	0.917	−0.381	0.000
D/L Glu	0.892	0.414	0.167
Proportion of variance	87.9	7.3	2.7

D-aIle/L-Ile: D-alloisoleucine/L-isoleucine; Leu: leucine; Asp: aspartic acid; Phe: phenylalanine; Glu: glutamic acid.

D/L ratios and age (Goodfriend, 1991; Collins et al., 1999). Nevertheless, the best correlations between D/L ratios of different amino acids (leucine, isoleucine, aspartic acid, phenylalanine and glutamic acid) of terrestrial gastropods ranging from present to Lower Pleistocene (D/L ratios up to 1.0), and time and/or the square root of time, were obtained with FOK (Torres et al., 1997).

Mitterer and Kriausakul (1989) modelled the racemization/epimerization reactions, at least for the epimerization of mollusks and for the early diagenetic history of fossils, in terms of apparent parabolic kinetics (APK), a procedure that generates a linear relationship between the square root of time and D/L ratios. However, the applicability of the parabolic approach at very low D-aIle/L-Ile ratios may be questionable. Likewise, for advanced stages of racemization/epimerization (high D/L ratios), the fitting to the empirical data is poor (Mitterer and Kriausakul, 1989; Murray-Wallace and Kimber, 1993).

Later, other authors applied APK and reported evidence for a linear relationship between the square root of time and D-aIle/L-Ile ratios base on data sets spanning a considerable period of geological age (Murray-Wallace and Kimber (1993) in fossil mollusks with D-aIle/L-Ile values up to 0.93; Hearty and Kaufman (2000) in marine oolitic and skeletal samples with D-aIle/L-Ile values up to 0.76; Oches and McCoy (2000) in terrestrial gastropods with D-aIle/L-Ile values up to 0.4). For other amino acids, the APK has also been applied, i.e. the extent of racemization of leucine (maximum D/L value: 0.83) and valine (maximum D/L value: 0.83) in molluskan fossils of Late Quaternary age (up to 225 ka) from southern Australia was described using APK (Murray-Wallace and Kimber, 1993). Similarly Oviatt et al. (1999) linear regressed ostracode D/L asp values (up to 0.55) with the square root of time.

Thereafter, other mathematical approaches have been reported: linear relationships have been obtained between time and D/L asp values (up to 0.27) of corals (Goodfriend et al., 1992); between time and a third power of D/L asp values (up to 0.11) of a marine gastropod (Goodfriend et al., 1995); between time and D-aIle/L-Ile values (up to 0.24) measured in land snail shells (Ellis et al., 1996); between time and racemization ratios, with values up to ca 0.7, of several amino acids (histidine, phenylalanine, aspartic acid, alanine, isoleucine, valine and glutamic acid) of fossil bones (Csapò et al., 1998); between time and a power of the function (1+D/L)/(1−D/L), D/L being either aspartic acid or glutamic acid measured in pyrolized and fossil ostracodes, with values up to 0.59 (Kaufman, 2000); between time and power of the function (1+D/L)/(1−D/L)D/L, D/L being the aspartic acid values in pyrolized and fossil marine mollusks, with values up to 0.78 (Manley et al., 2000).

In conclusion, "no model appears to satisfactorily describe the patterns of each of the amino acids. Consequently, a model must be chosen empirically for each data set based on the goodness of fit" (Goodfriend, 1991). In fact, in the study of Goodfriend (1991), using samples for the same time range, the patterns of some amino acids better linearized with APK while for others the "first-order kinetics" trend were the best one.

The ostracode mean D/L ratios of each locality and the numerical dating were obtained using U/Th, paleomagnetism, amino acid racemization and radiocarbon methods employed in this work are in Table 4.

In order to select the best trend, we have compared the correlation coefficients (r) for different approaches. According to these results, we have chosen to work with FOK transformation of the isoleucine, leucine, phenylalanine and glutamic acid D/L ratios vs. time and the first-order kinetic transformation of the aspartic acid D/L ratios vs. square root of time due to the high correlation coefficients (Table 5) although in two cases (isoleucine and phenylalanine) the APK approach seems to have slightly higher r values (only 0.02 units). In these latter two cases, the reason to work with FOK approach is because for very old materials there is a major deviation from the predicted degree of racemization based on the kinetic model and the true age (Mitterer and Kriausakul, 1989; Murray-Wallace and Kimber, 1993). The racemization algorithms used are based on those proposed by Goodfriend and Mitterer (1988) and Goodfriend (1991), which were modified from those of Bada and Protsch (1973) and Mitterer (1975), with the adjustment to time or the square root of time (Torres et al., 1997). For the amino acids isoleucine, leucine, phenylalanine and glutamic acid, the best fit is obtained with respect to time instead of reporting the data in the context of the square root of time, as for aspartic acid. The results are (Fig. 4):

For isoleucine:

$$t = 106.67 + 370.04\ \mathrm{Ln}\left[\frac{0.565}{0.565 - \dfrac{D\text{-aIle/L-Ile}}{1 + D\text{-aIle/L-Ile}}}\right],$$

$$r = 0.945, \quad p = 0.000.$$

For leucine:

$$t = -0.8918 + 486.20\ \mathrm{Ln}\left[\frac{1 + \mathrm{D/L}}{1 - \mathrm{D/L}}\right],$$

$$r = 0.957, \quad p = 0.000.$$

For aspartic acid:

$$\sqrt{t} = -2.666 + 18.027\ \mathrm{Ln}\left[\frac{1 + \mathrm{D/L}}{1 - \mathrm{D/L}}\right],$$

$$r = 0.991, \quad p = 0.000.$$

Table 4
Ages and amino acid racemization ratios analysed in ostracodes of the different localities used in the age calculation algorithms

Locality	Age (ka)	D-aIle/L-Ile	D/L Leu	D/L Asp	D/L Phe	D/L Glu	*n*
CGS	0 (this work)	—	—	0.074±0.009	—	0.023±0.001	3
SPD-0198	6,782±0.120 ^{14}C (this work)	—	0.025±0.005	0.182±0.000	0.086±0.032	0.056±0.001	2
PR-6	105.132±7.648 U/Th (Torres et al., 1994)	—	—	0.333	0.251	0.117	1
PR-7	156.005±7.970 U/Th (Torres et al., 1994)	0.186±0.004	0.203±0.022	0.375±0.001	0.276±0.037	0.122±0.008	2
SDP-3160	167.321±17.659 U/Th (this work)	0.183±0.000	0.258±0.000	0.401±0.010	0.306±0.000	0.140±0.005	2
FA	337±15 Aard (Ortiz et al., 2000)	0.419	0.376	0.587	0.373	0.364	1
CBS-323	ca 412 Pm (Ortiz, 2000)	0.529±0.106	0.397±0.098	0.522±0.016	0.429±0.085	0.397±0.031	7
CB	476±24 Aard (Ortiz et al., 2000)	0.559±0.040	0.479±0.039	0.575±0.005	0.485±0.074	0.373±0.010	8
CBS-268	ca 760 Pm (Ortiz, 2000)	0.736±0.055	0.544±0.055	0.683±0.040	0.519±0.094	0.579±0.100	6
CBS-253	ca 800 Pm (Ortiz, 2000)	0.725±0.046	0.570±0.061	0.700±0.013	0.502±0.035	0.534±0.042	5
VM	1095±55 Aard (Ortiz et al., 2000)	1.156±0.057	0.848±0.004	0.756±0.011	0.850±0.062	0.720±0.010	3

Pm: paleomagnetism; aard: amino acid racemization dating; D-aIle/L-Ile: D-alloisoleucine/L-isoleucine; Leu: leucine; Asp: aspartic acid; Phe: phenylalanine; Glu: glutamic acid; *n*: number of samples analysed from each stratigraphic horizon. In SPD-0198, SPD-3160, PR-6 and PR-7 localities ostracode valves belong to *Herpetocypris reptans* (Baird); in VM locality ostracode valves belong to Cyprideis torosa (Jones) and *Ilyocypris bradyi* Sars. In CGS, FA, CB, CBS-323, CBS-268, CBS-253 localities ostracodes belong to *Cyprideis torosa* (Jones).

Table 5
Correlation coefficients (*r*) between time and D/L ratios of amino acids

	1	2	3
D-aIle/L-Ile	0.945	0.913	0.976
D/L Leu	0.957	0.915	0.967
D/L Asp	0.952	0.991	0.986
D/L Phe	0.916	0.872	0.947
D/L Glu	0.988	0.952	0.967

D-aIle/L-Ile: D-alloisoleucine/L-isoleucine; Leu: leucine; Asp: aspartic acid; Phe: phenylalanine; Glu: glutamic acid.
Correlations are presented for: (1) D/L ratios transformed to first-order kinetics vs. time; (2) D/L ratios transformed to first-order kinetics vs. square root of time; (3) untransformed D/L ratios vs. square root of time (APK). All correlations are statistically significant at the level of $p<0.001$.

For phenylalanine:

$$t = -51.80 + 513.59\ \mathrm{Ln}\left[\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right],$$

$$r = 0.916, \quad p = 0.000.$$

For glutamic acid:

$$t = -39.59 + 622.25\ \mathrm{Ln}\left[\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right],$$

$$r = 0.988, \quad p = 0.000.$$

These algorithms will be adequate for age calculation of ostracode samples in the Iberian Peninsula from the Lower Pleistocene to the present. However, for young samples there are two reasons why we prefer to use other algorithms:

(1) The model of the racemization process (Goodfriend and Mitterer, 1988; Goodfriend, 1991) employed in this work consists on the combination of two functions with different slopes (because of the "non-linear" behaviour of the racemization process). Moreover, for the calculation of these algorithms, we have used dated samples of a wide range of ages (from 0 yr to ca 1 Ma). Consequently, it is our view that other algorithms are better suited for use with young samples.
(2) In addition, racemization is genus-dependent, and it seems that even more in young samples (for old

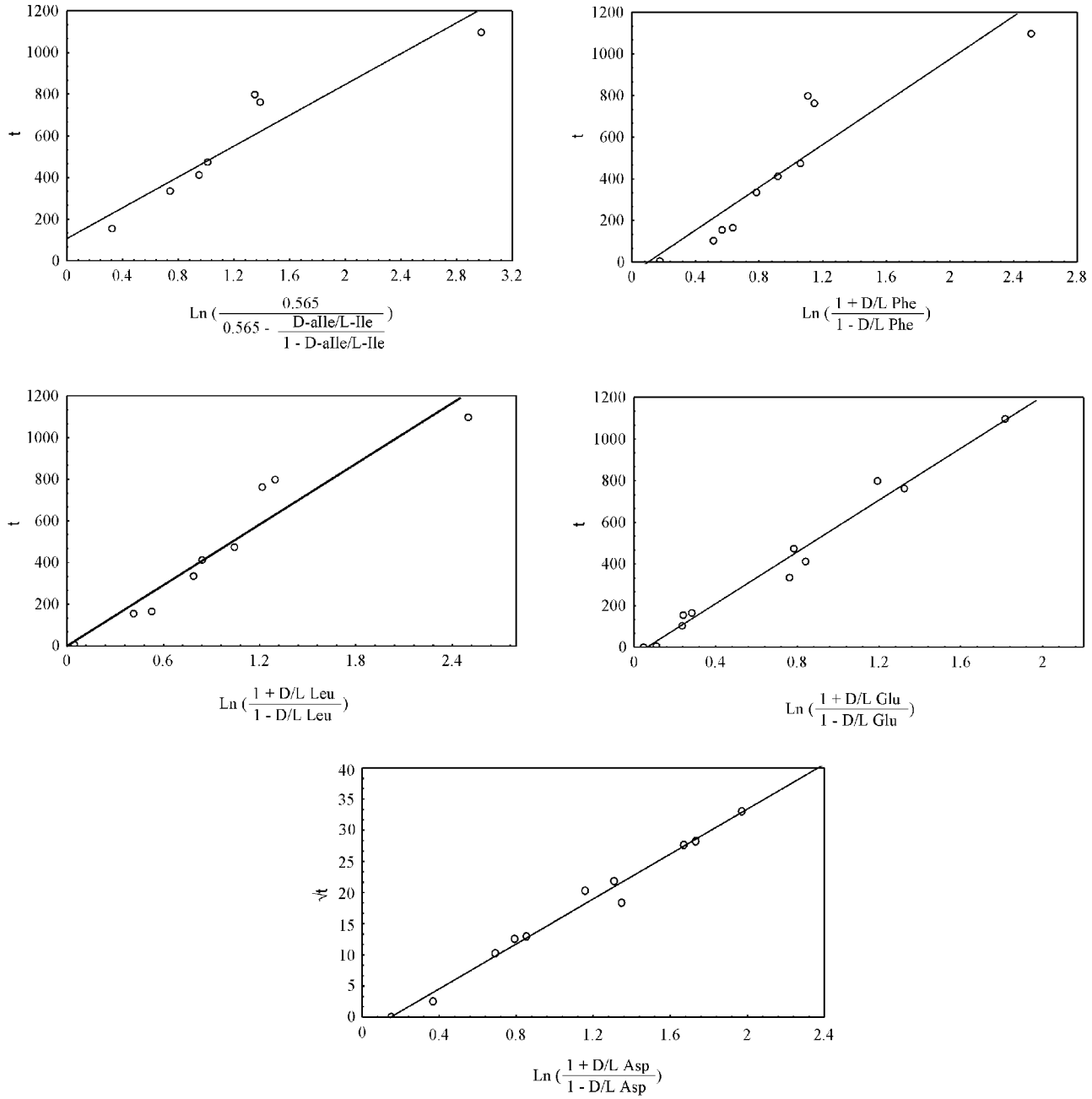

Fig. 4. Plots of first-order kinetics transformed D/L ratios of ostracodes vs. time (for isoleucine, leucine, phenylalanine and glutamic acid) and vs. square root of time (for aspartic acid). D-aIle/L-Ile: D-alloisoleucine/L-isoleucine; Leu: leucine; Asp: aspartic acid; Phe: phenylalanine; Glu: glutamic acid.

samples the racemization ratios become similar) (Torres et al., 2000).

In view of these considerations, we also present the calculation of models for samples containing only *Herpetocypris reptans* (Baird). These are the samples with the lowest measured D/L ratios. In this case, only equations for D/L aspartic acid, phenylalanine and glutamic acid were calculated (Table 4) due to the limited results for isoleucine and leucine and, their poor reproducibility. Likewise, according to Torres et al. (2000) these amino acids present enough reliability for the age calculation of young samples.

For this purpose, age calculation algorithms were defined using the models proposed by Torres et al. (1997), modified from Goodfriend and Mitterer (1988) and Goodfriend (1991), with the adjustment to the square root of time. This was based on the comparison between the correlation coefficients obtained with different approaches (Table 6). Similar correlation

Table 6
Correlation coefficients (r) between time and D/L ratios of amino acids measured in localities with *Herpetocypris reptans* Sars ostracodes (SPD-0198, SPD-3160, PR-6 and PR-7)

	1	2	3
D/L Asp	0.966	0.993	0.992
D/L Phe	0.983	0.995	0.995
D/L Glu	0.960	0.989	0.989

Asp: aspartic acid; Phe: phenylalanine; Glu: glutamic acid.
Correlations are presented for: (1) D/L ratios transformed to first-order kinetics vs. time; (2) D/L ratios transformed to first-order kinetics vs. square root of time; (3) untransformed D/L ratios vs. square root of time (APK). All correlations are statistically significant at the level of $p<0.005$.

coefficients were obtained applying the FOK transformation of D/L ratios vs. the square root of time and the APK model. In this case we chose the former approaches because for low D/L ratios the applicability of the APK may be questionable (Mitterer and Kriasusaul, 1989). The results are (Fig. 5):

For aspartic acid:

$$\sqrt{t} = -3.586 + 19.745 \ \mathrm{Ln}\left[\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right],$$

$$r = 0.993, \quad p = 0.001.$$

For phenylalanine:

$$\sqrt{t} = -1.380 + 23.246 \ \mathrm{Ln}\left[\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right],$$

$$r = 0.995, \quad p = 0.005.$$

For glutamic acid:

$$\sqrt{t} = -3.186 + 58.972 \ \mathrm{Ln}\left[\frac{1+\mathrm{D/L}}{1-\mathrm{D/L}}\right],$$

$$r = 0.989, \quad p = 0.001.$$

These equations can only be applied in samples with racemization ratios below those measured in SPD-3160 level (D/L Asp: 0.401 ± 0.010; D/L Phe: 0.306 ± 0.000; D/L Glu: 0.140 ± 0.005).

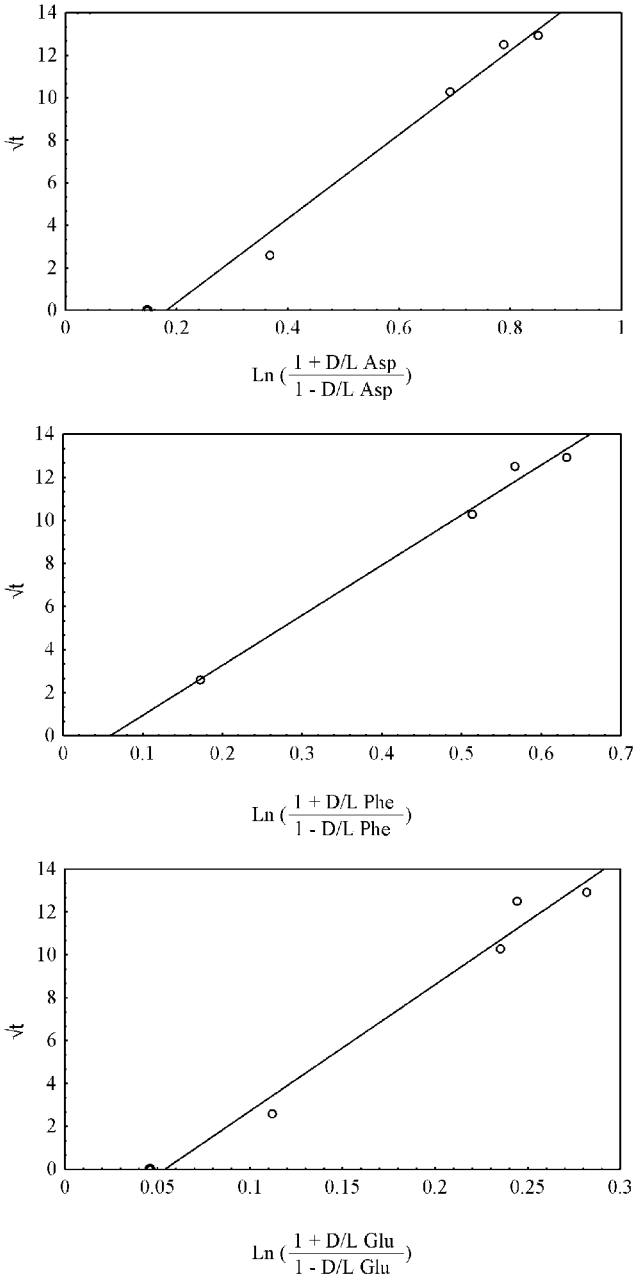

Fig. 5. Plots of first-order kinetics transformed D/L ratios of ostracodes vs. square root of time. Asp: aspartic acid; Phe: phenylalanine; Glu: glutamic acid.

5. Aminochronology of the Guadix-Baza Basin

With the aid of these algorithms, the aminochronology of the general GBE Basin "composite-stratotype-section" has been established. For this purpose, amino acid racemization results obtained in ostracodes were combined with previously obtained paleomagnetism results.

Samples recovered from the bottom of the stratigraphic section show a paleomagnetic polarity change from normal to reverse in 18 m (Fig. 3), which was interpreted (Ortiz, 2000) as the end of the Olduvai chron (i.e., the Plio–Pleistocene boundary) established at 1.77 Ma (Cande and Kent, 1995).

Numerical dating is obtained by introducing in the algorithms the ostracode D/L ratios (*C. torosa* in all cases) of each amino acid for the different localities. The age of a single stratigraphic horizon is the average of the numerical datings obtained for each amino acid D/L ratios measured in samples of that unit (Table 7). The age uncertainty of a stratigraphic horizon is the standard deviation of all the numerical ages calculated from the amino acid D/L ratios measured in the samples of each level.

Table 7
Summary of the datings in the Guadix-Baza Basin stratrigraphic section obtained by the amino acid racemization method in ostracodes and paleomagnetism (pm)

Level	m	D-aIle/L-Ile	D/L Leu	D/L Asp	D/L Phe	D/L Glu	Age	*n*
CBS-206	206.5	0.997±0.021	0.710±0.030	0.780±0.066	0.805±0.007	0.703±0.083	1012±169	3
CBS-223	223.8	1.045±0.035	0.735±0.007	0.820±0.014	0.780±0.000	0.725±0.092	1008±125	2
CBS-228	228.1	0.819±0.000	0.828±0.000	0.711±0.000	0.678±0.000	0.594±0.000	863±173	
CBS-253	253.0	0.725±0.046	0.570±0.061	0.700±0.013	0.502±0.035	0.534±0.042	ca 800 (pm) 743±88	5
CBS-268	269.3	0.736±0.055	0.544±0.055	0.683±0.040	0.519±0.094	0.579±0.100	ca 760 (pm) 736±115	6
CBS-281	281.2	0.877±0.046	0.603±0.031	0.687±0.004	0.603±0.029	0.599±0.007	737±68	3
CBS-303	303.4	0.675±0.021	0.490±0.014	0.605±0.021	0.435±0.007	0.445±0.007	517±56	2
CBS-314	314.0	0.520±0.071	0.373±0.051	0.534±0.006	0.436±0.099	0.376±0.037	407±58	5
CBS-323	323.3	0.529±0.106	0.397±0.098	0.522±0.016	0.429±0.085	0.397±0.031	ca 412–419 (pm) 409±95	7
CBS-327	327.1	0.492±0.011	0.397±0.031	0.492±0.004	0.430±0.040	0.342±0.015	389±62	3
CBS-330	329.8	0.395±0.037	0.336±0.001	0.481±0.054	0.320±0.000	0.40±0.000	339±68	3
CSU-352	352.5	0.332±0.001	0.279±0.081	0.441±0.019	0.346±0.051	0.325±0.005	279±77	3

D-aIle/L-Ile: D-alloisoleucine/L-isoleucine; Leu: leucine; Asp: aspartic acid; Phe: phenylalanine; Glu: glutamic acid; *n*: number of samples analysed from each stratigraphic horizon. In all samples ostracode valves belonged to the species *Cyprideis torosa* (Jones).

It can be observed that the Matuyama/Brunhes magnetozone boundary (ca 780 ka) located at around 260 m of the section (Oms et al., 1994; Ortiz, 2000) was correctly interpreted. In fact, samples CBS-253 and CBS-268 were dated at 743±88 and at 736±15 ka, respectively.

Also the ages of CBS-323 (409±95 ka) and CBS-314 (407±50 ka) are in accordance with the reverse polarity event located between 314 and 323 m, which indicated the presence of either Emperor (419 ka) or Lake Biwa III (412 ka) excursions (Ortiz, 2000). The top of the GBE Basin section has been dated at 279±77 ka.

With the aid of the paleomagnetism and amino acid racemization datings, the Pleistocene mean sedimentation rate for the Guadix-Baza Basin was calculated. For this purpose two section horizons were chosen: the first, sample CBS-18, where we identified the end of Olduvai Chron (Plio–Pleistocene boundary) at 1.77 Ma and the second, sample CBS-352, close the top of the section dated at 279±77 ka. The resulting mean Pleistocene sedimentation rate for the Guadix-Baza Basin was $S_r = 4.464$ ka/m.

A low subsidence rate can be assumed for the Norte de Orce sub-section given the shallowness of the basement composed of Jurassic limestones. In this sub-section a partial sedimentation rate was calculated: $S_r = 6.055$ ka/m (between samples CBS-268 and CBS-323). The partial sedimentation rate of the uppermost part was also calculated: $S_r = 4.586$ ka/m (between samples CBS-323 and CBS-352).

Both results are lower than the mean Pleistocene sedimentation rate for the Guadix-Baza Basin. A lowering of the sedimentation rate towards the end of the basin infill cannot be ruled out. However, when the position of the stratigraphic horizons of the section are potted vs. their datings (Fig. 6) it is possible to observe that there is a linear relationship. This is due to the fact that the lack of an important compaction in the dominantly sandy lower part of the section is negligible at its uppermost, more lutitic, part.

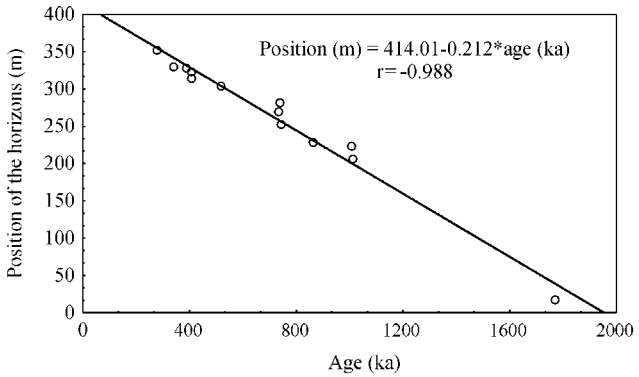

Fig. 6. Plot of the position (m) from bottom to top of the stratigraphic horizons of GBE Basin representative stratigraphic section vs. their datings (ka).

6. Conclusion

Age calculation algorithms for D/L ratios of five amino acids (isoleucine, leucine, aspartic acid, phenylalanine and glutamic acid) analysed in continental ostracodes were determined for southern and central Iberian Peninsula. These algorithms allow for the numerical dating of deposits from zones with a similar thermal history (i.e. in the Mediterranean area) from the Lower Pleistocene to present.

With the aid of these algorithms, together with paleomagnetism, the chronostratigraphy for the Pleistocene record of the Guadix-Baza Basin was obtained based on a representative 356-m-thick basin section (presented in Fig. 3). An estimate of the mean sedimentation rate of this basin for the Pleistocene ($S_r = 4.464$ ka/m) was also calculated.

In order to obtain more accurate results for young samples other algorithms were calculated for aspartic acid, phenylalanine and glutamic acid.

It has been shown that ostracodes are a powerful tool for amino acid dating purposes. This is not only due to their presence in a large number of beds, but also to the geochemistry of their valves and the high preservation of amino acids in their caparaces, even in very old samples.

These results suggest that the range of the amino acid racemization dating method in the Iberian Peninsula is older than 1.1 Ma as Torres et al. (1994) based on the results of D/L ratios analysed in gastropods suggested, beign ca 1.3–1.4 Ma.

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